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13. ABSTRACT (Maximum 200 Words)

Synucleins (a, b, g, synoretin) are a family of small, highly conserved proteins expressed predominantly in neurons. While a-synuclein is implicated in neurodegenerative diseases, g-synuclein is expressed in the majority (>85%) of late-stage breast and ovarian carcinomas and is not expressed in normal mammary and ovarian epithelium. In spite of their significance, the normal and pathological roles of synucleins are not fully understood. To address the biological function of g-synuclein and its role in the malignancy of breast and ovarian cancer, we ectopically over-expressed g-synuclein in several cancer cell lines. Recently we found that g-synuclein is associated with two major mitogen-activated kinases (MAPK), i.e., extracellular signal-regulated protein kinases (ERK 1/2) and c-Jun N-terminal kinase 1 (JNK1). Over-expression of g-synuclein lead to constitutive activation of ERK1/2, and down-regulation of JNK1 in response to stress (UV, sodium arsenate, and heat shock). In this study, we further characterized the effects of g-synuclein on paclitaxel, a commonly used chemotherapeutic drug, and nitric oxide induced apotosis. We found that g-synuclein over-expressing cells were more resistant (4- to 5-fold) to paclitaxel or nitric oxided as compared to the parental cells. This resistance to paclitaxel could be partially restored when ERK activity was inhibited using U0126, a MEK1/2 inhibitor. In addition, activation of the mitochodrial apoptotic pathway (JNK and/or caspase 3 activation) by paclitaxel and nitric oxide was blocked by ectopic expression of g-synuclein. Collectively, these data indicate that g-synuclein may be involved in the pathogenesis of breast and ovarian cancer by promoting tumor cell survival under adverse conditions and by providing resistance to certain anti-cancer drugs. Because of its high frequency of expression in late-stage breast and ovarian cancers, g-synuclein may be a promising target for therapy.

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INTRODUCTION:

The synucleins (α, β, γ, synoretin) are a family of highly conserved small proteins that are normally expressed predominantly in neurons. Little is known about the normal functions of synucleins in physiological conditions. Of the synucleins, α-synuclein is the best characterized because of its potential significance in neurodegenerative diseases including Parkinson s Disease. Recently we and others have found that γ-synuclein is dramatically up-regulated in the vast majority of late-stage breast (70%) and ovarian (> 85%) cancers and that γ-synuclein over-expression can enhance tumorigenicity (Bruening et al., 2000; Ji et al., 1997; Liu et al., 2000). We also observed that expression of γ-synuclein induces a phenotype similar to that induced by activation of RhoA/Rac/CDC42, altering the appearance of focal adhesions and stress fibers, and enhancing motility and invasion in ovarian cancer cells. Recent studies by Ji and colleagues have also shown that when γ-synuclein is overexpressed in a breast tumor derived cell line, the cells experience a dramatic augmentation in their capacity to metastasize *in vivo* (Jia et al., 1999). Based on these known data, we hypothesized that γ-synuclein may be a proto-oncogene, and that the abnormal expression of this protein (i.e., oncogenic form) in breast and ovarian tumors may contribute to the metastatic spread and high morbidity associated with advance stages of these diseases. To address this hypothesis, we first ectopically over-expressed γ-synuclein in several cancer cell lines and the effects of γ-synuclein on the phenotypes of these cells were charaterized. The mechanisms underlying γ-synuclein induced cell phenotype changes were investigated by biochemical and cellular assays. We found that γ-synuclein is associated with two major mitogen-activated kinases (MAPK), i.e., extracellular signal-regulated protein kinases (ERK1/2) and c-Jun N-terminal kinase 1 (JNK1). Over-expression of γ-synuclein may enhance cell motility by activating Rho/Rac/Cdc42 and ERK pathways. JNK activation induced by stress (UV, arsenate, and heat shock) and chemotherapy drugs (Taxol, vinblastine) can also be down-regulated by γ-synuclein. Finally, we found that γ-synuclein can protect chemotherapy drug-induced cell death by modulating the ERK cell survival pathway and the JNK-mitochondria-Caspase9/3 pathway.

Body.

γ-Synuclein interacts with ERK and JNK MAP kinases in cancer cells - α-Synuclein has recently been reported to bind directly to the ERK2 kinase (Iwata et al., 2001b). Therefore, we evaluated whether γ-synuclein could also interact with the ERK kinases as well as other MAPKs. By co-immunoprecipitation
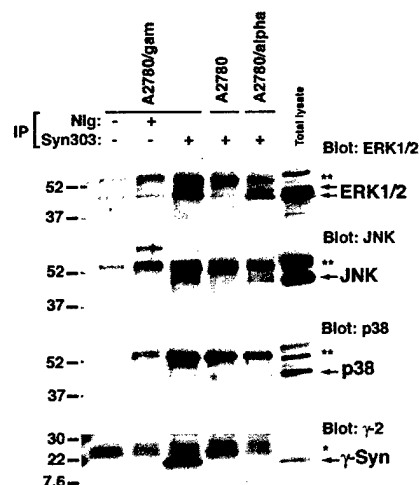

Fig. 1. Interaction between γ- and α-synucleins with ERK1/2 and JNK. Cell lysate from A2780, A2780/gam or A2780/alpha were immunoprecipitated with Syn303, NIg (normal IgG) or irrelevant antibodies (not shown here). The proteins in the immunoprecipitates were identified by immunoblotting with antibodies against ERK1/2, JNK1, p38, and γ-2 (a polyclonal antibody specific for γ-synuclein). Molecular mass standards (in kilodaltons) are indicated on the left. Non-specific bands around the IgG heavy (**) and light (*) chains are indicated by asterisks.

approaches, we were able to demonstrate a novel association of γ-synucleins with ERK1/2 and JNK1 kinase, but not with the p38 kinase (**Fig. 1**). We also confirmed that α-synuclein is associated with ERK1/2 as well with JNK1 (**Fig. 1**), which is consistent with the recently studies using neuro2a, a

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neuronal cell line (Iwata et al., 2001a). These data indicate that γ- and α-synuclein can interact with ERK1/2 and JNK1 in cancer cells that over-express these proteins.

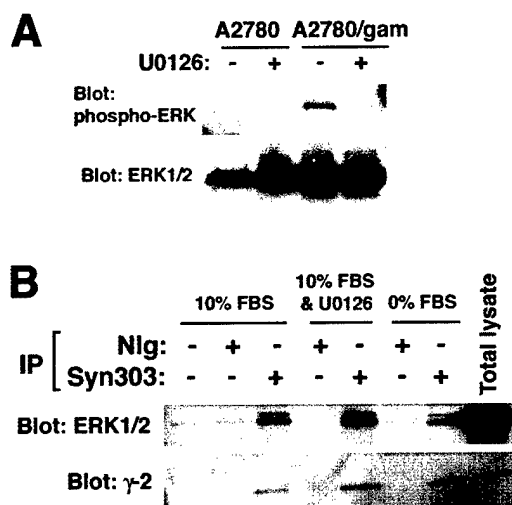*Elevated activity of ERK in cells over-expressing γ-synuclein* - We next evaluated whether these protein interactions would affect the activity of ERK1/2 and/or JNK1 (see below). In A2780 and OVCAR5 cancer cells over-expressing γ-synuclein, the activated ERK1/2 was increased 2-3 fold as evidenced by immunoblotting with an anti-phospho-ERK specific antibody (**Fig. 2**). In contrast, α- and β-synucleins appeared to have little or no effect on the activity of ERK1/2 (**Fig. 2A**) although α-synuclein was also found to be associated with ERK (as described above and shown in **Fig. 1**) in A2780 cells. In HEK 293 cells, the basal level of ERK activation is undetectable and γ-synuclein over-expression does not increase its activation level (**Fig. 2B**). Structural analysis indicate that γ-synuclein does not contain any kinase domain, suggesting that the activation of ERK is mediated by other kinase. Since MEK1/2 is required for the activation of ERK1/2 in response to many mitogens, we determined whether MEK1/2 is still required
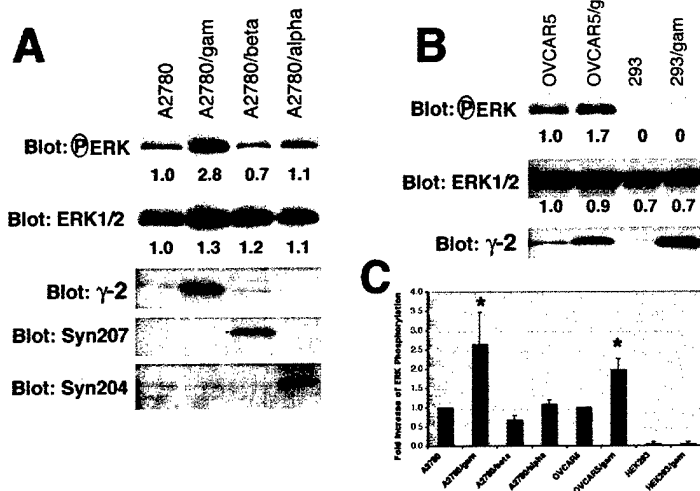
Fig. 2. Activation of ERK in cells over-expressing γ-synuclein. A, ERK1/2 activation is enhanced in γ-synuclein over-expressing A2780 cells. Whole cell lysates (40 g/lane) from A2780 cells (parent) or A2780 cells transfected with γ-, β-, or α-synuclein were separated by SDS-PAGE and blotted with appropriate antibodies. The levels of activated ERK or total ERK1/2 were determined using an anti-phospho-ERK specific antibody or ERK1/2 antibodies, respectively. The number beneath each band represents the arbitrary densitometry units of the corresponding band (the integrated densitometry reading number of A2780 or OVCAR5 was assigned to an arbitrary unit 1.0, and the other readings were normalized thereafter). The synucleins were evaluated by blotting with specific antibodies, i.e., γ-2 for γ-synuclein, Syn207 for β-synuclein, and Syn204 for α-synuclein, respectively. B, activation of ERK by γ-synuclein in OVCAR5 cells but not HEK 293 cells. Whole cell lysate (40 μg/lane) from parental or γ-synuclein over-expressing cells were separated and blotted as in A. Panels A and B are representative of at least three independent experiments with comparable results. C, fold increase of ERK activation. The data shown are the average – S.E. of three independent experiments. Phosphorylated ERK was normalized to the protein level of total ERK. The basal levels of ERK phosphorylation in the parental A2780 or OVCAR5 cells were set as 1.0. (*) Represents significant difference compared to the parental cells (p < 0.05).

for γ-synuclein mediated activation of ERK1/2. When cells over-expressing γ-synuclein were treated with the MEK1/2 inhibitor U0126, the activation of ERK1/2 was suppressed (**Fig. 3A**). We further studied the relation of γ-synuclein-ERK interaction and the activation status of ERK1/2. In cells treated with U0126 or serum-starved, the association of γ-synuclein and ERK1/2 was still present (**Fig. 3B**). These data indicate that γ-synuclein may be constitutively associated with ERK1/2, which could facilitate the activation of ERK by MEK1/2 and lead to the constitutive activation of ERK1/2 in cells over-expressing γ-synuclein.

A

A2780   A2780/gam

U0126: - + - +

Blot:
phospho-ERK

Blot: ERK1/2

B

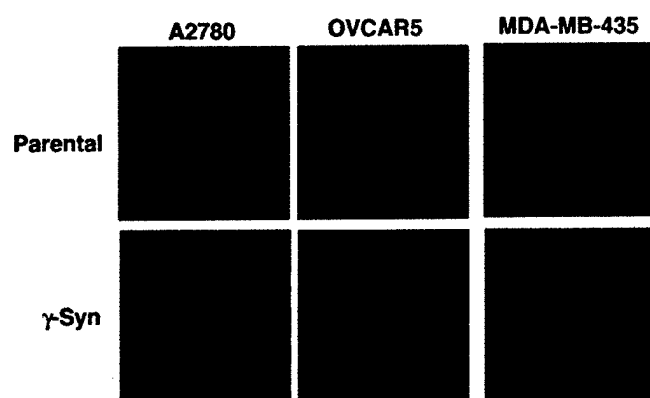|		10% FBS		10% FBS & U0126		0% FBS		Total lysate
IP { Nlg:	-	+	-	+	-	+	-	
Syn303:	-	-	+	-	+	-	+	

Blot: ERK1/2

Blot: γ-2

Fig. 3. Requirement of MEK1/2 for γ-synuclein enhanced ERK1/2 activation. A, A2780 and A2780/gam cells untreated or treated with the MEK1/2 inhibitor, U0126 (10 μM), were lysed and 30 μg of proteins were loaded into each lane. As in Fig. 2, anti- phospho-ERK1/2 specific antibody was used to detect activated ERK1/2; and the antibody against ERK1/2 was used to detect the protein level of total ERK1/2. B, the interaction between ERK and γ-synuclein is independent of the activation status of ERK1/2. A2780/gam cells in normal 10% FBS medium, 10% FBS medium with U0126 (10 μM), or serum-free medium were lysed and immunoprecipitated with Syn303 or control IgG. The proteins in the immunoprecipitates were detected with the antibodies against ERK1/2 or against human γ-synuclein (γ-2). The autoradiogram shown is the representative of three independent experiments with comparable results.

Over-exprssion of γ-synuclein leads to increased cell motility- Recent stuidies by Jia and colleagues indicated that γ-synuclein may increase cell migration and metastasis (Jia et al., 1999). We also established several stable cancer cell lines that over-express γ-synuclein (**Fig. 4A,** and data not shown) and found that γ-synuclein can enhance cell motility as analysed by Boyden chamber assay (**Fig. 4B, 4C, 4D,** and data not shown). Consistent with their role in cell migration, more stress fibers were found in cells over-expressing γ-synuclein (**Fig. 5**)
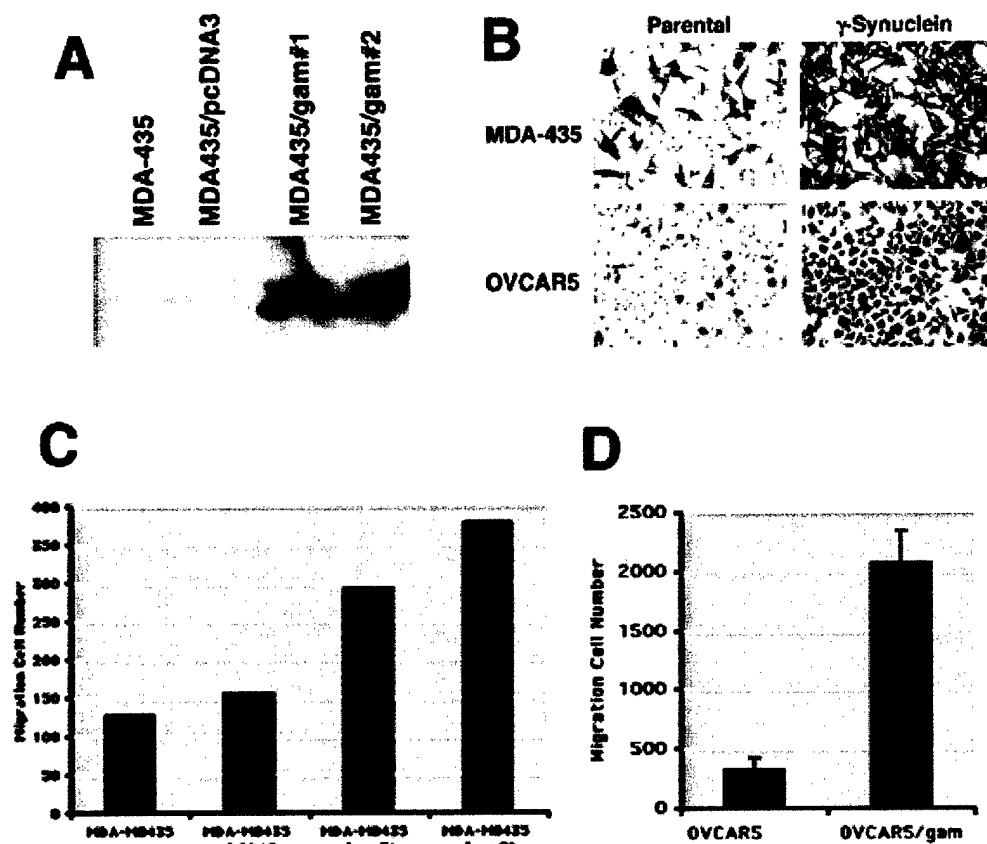
**Fig.4.** γ-Synuclein enhance cell migration. A, γ-synuclein expression in parental MDA-MB-435 cells, and those stably transfected with pcDNA3 vector alone, or with CMV-γ-synuclein (two clones were shown here). B throuhg D, Boyden chamber assay for cell migration. Cells migrated to the lower chamber were stained (B) and counted (C, D).

Over-exprssion of γ-synuclein leads to activation of at least one member of the Rho family GTPase- Cell migration and invasion involves a series of coordinated complex processes, including focal adhesion formation in the front and release of adhesion in the back, polarized stress fiber formation and disassembly and contraction (Ridley, 2000). Several protein kinases are known to regulate these processes, including Rho/Rac/Cdc42 small GTPase proteins, ERK, Crk, and Akt (Krueger et al., 2001; Ridley, 2001; Ridley et al., 1999; Wicki and Niggli, 2001). Among these effector kinases, Rho family members play a pivotal role in regulating stress fiber and focal adhesion formation. In cells over-expressing γ-synuclein, we found at least one major Rho/Rac/Cdc42 member is activated although the protein levels of these proteins were not affected (Fig. 6).
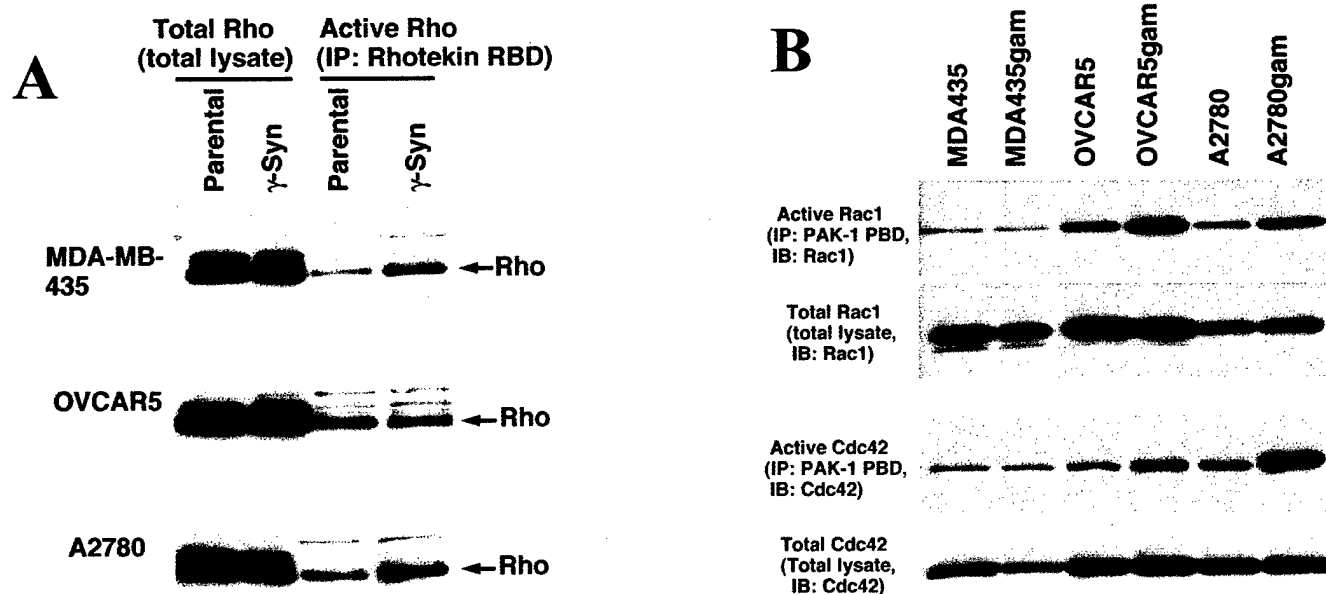
Fig. 6. Enhanced activation of Rho (A) and Rac/Cdc42 (B) in cancer cells over-expressing γ-synuclein. A, whol3 cell lysate (2 mg protein) were immunoprecipitated with Rhotekin Rho binding domain and analyzed by Western blot with anti-Rho antibody. B, who cell lysate (2 mg protein) were immunoprecipitated with PAK1 PBD and analyzed by Western blot with anti-Rac and anti-Cdc42 antibodies, respectively.
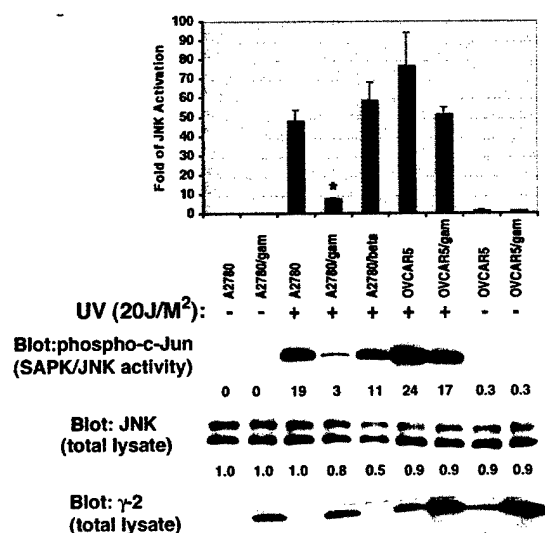
Fig. 7. Inhibition of JNK activation by γ-synuclein in response to UV treatment. A2780, A2780/gam, A2780/beta, OVCAR5, OVCAR5/gam cells were un-treated or treated with UV (20 J/M^2) and cells were lysed at 30 min. JNK activities were analyzed by an immunocomplex kinase assay using GST-c-JUN as substrate. The phosphorylated GST-c-JUN by activated JNK was evaluated by immunoblotting with anti-phospho-c-JUN specific antibody. The protein levels of JNK and γ-synuclein were determined by immunoblotting with anti-JNK and γ-2 antibody, respectively. The number beneath each band represents the arbitrary densitometry units of the corresponding band. The blots shown here are representative of three independent experiments with comparable results. JNK activities were measured by levels of phosphorylated c-JUN and normalized to levels of total JNK proteins. The graph above the blots is the average ± S.E. of three independent experiments. (*) Represents significant inhibition of JNK activation compared to that in the parental cells (p < 0.05).
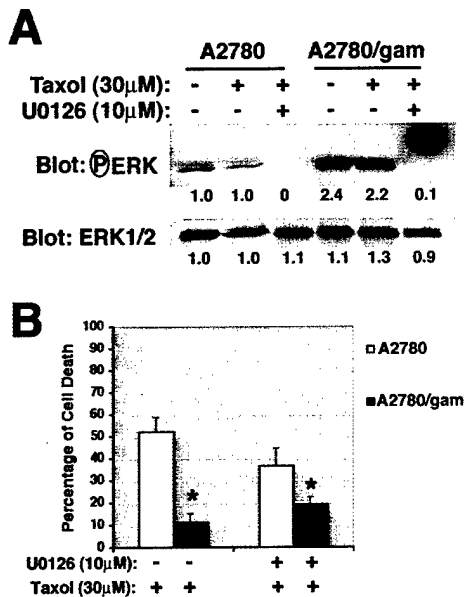
Down-regulation of JNK activation by γ-synuclein in response to UV - JNK is activated by stress signals including UV which leads to mitochondria mediated apoptosis (Davis, 2000). The basal level of JNK activity in A2780 and OVCAR5 ovarian cancer cells is very low in untreated cells whether γ-synuclein was over-expressed or not (**Fig. 7**). JNK was highly activated in the parental cells when treated with UV (**Fig. 7**). In cells over-expressing γ-synuclein, the activation of JNK was almost completely blocked in A2780/gam cells (p < 0.05) and was down-regulated by approximately 50% in OVCAR5/gam cells when treated with UV (**Fig. 7**) or heat-shock (data not shown). The blockage of JNK activation by UV appears to be γ-synuclein specific since over-expression of α- and β-synucleins appeared to have little or no effect on the activation of JNK by UV (**Fig. 7,** and data not shown). The inhibition of JNK activation in OVCAR5/gam cells is much less than that in A2780/gam cells. The cause of this difference could either be cell-type specific, or it could be the high endogenous γ-synuclein expression in OVCAR5 cells. Collectively, these data indicate that stress-induced activation of JNK can be blocked by γ-synuclein over-expression in a variety of cell lines.

γ-Synuclein may protect paclitaxel (Taxol) induced cell death by regulating JNK and ERK activities.
Based on the data presented above, we hypothesized that γ-synuclein may contribute to cancer cell survival by up-regulating the ERK cell survival pathway and by suppressing the JNK apoptosis pathway under adverse conditions. However, when we treated γ-synuclein over-expressing cells with UV, significant differences in cell survival between A2780 and A2780/gam cells were not observed (data not shown). The reason for this lack of difference is not readily apparent. However, cell survival and cell death are regulated by the counter-balance between the survival factors and the apoptotic signaling pathways. Since UV treatment also activates the cell survival pathways ERK [(Rosette and Karin, 1996), and data not shown] and PI3K-AKT (Krasilnikov et al., 1999; Nomura et al., 2001), the initiation of the mitochondria associated caspase pathway may be blocked by the activation of ERK or AKT in A2780 cells. In support of these findings we did not observe the cleavage of the caspase-3 substrate PARP in A2780 cells when treated with UV (data not shown).

We next evaluated the survival of γ-synuclein over-expressing cells in response to Taxol, a commonly used chemotherapeutic drug. In addition to its role in affecting microtubule assembly, Taxol is known to lead to apoptosis via the mitochondria by activating the JNK signaling pathway and Taxol-induced apoptosis can be enhanced by MEK inhibition (Lee et al., 1998; Mandlekar et al., 2000; Wang et al., 1999; Wang et al., 2000). In A2780 cells, Taxol did not affect the basal level activity of ERK or the activation of ERK by γ-synuclein (**Fig. 8A**). To test the effect of γ-synuclein on cell survival, cells were treated with Taxol for varying lengths of time. At 48hr after treatment, 45-60% of A2780 cells had died, while only 7-15% of A2780/gam cells were dead indicating that Taxol
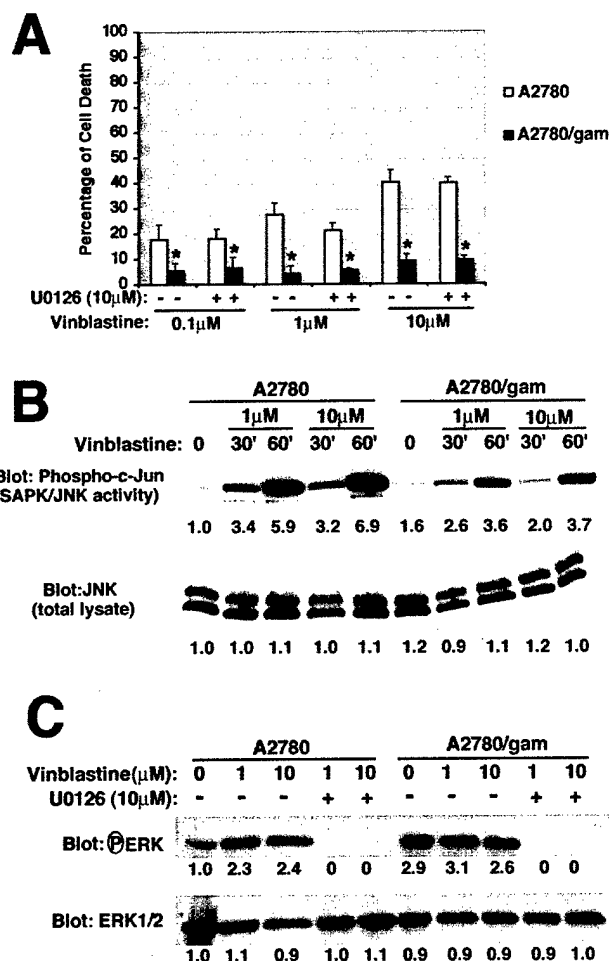
A

	A2780	A2780/gam
Taxol (30µM):	- + +	- + +
U0126 (10µM):	- - +	- - +

Blot: ℗ERK

1.0 1.0 0 2.4 2.2 0.1

Blot: ERK1/2

1.0 1.0 1.1 1.1 1.3 0.9

B

Percentage of Cell Death

□ A2780
■ A2780/gam

U0126 (10µM): -   -   +   +
Taxol (30µM): +   +   +   +

Fig. 8. γ-Synuclein protects cells from paclitaxel (Taxol) induced cell death and is partially mediated by ERK activation. A, Taxol does not affect ERK activity or γ-synuclein mediated ERK activation. A2780 or A2780/gam cells pre-treated with or without U0126 (10 µM) were treated with Taxol (30 µM) in the absence or presence of U0126 for 30 min. Whole cell lysate were separated by SDS-PAGE and blotted with phospho-ERK to determine the level of active ERK, or with ERK1/2 antibodies for the level of total ERK1/2. The number beneath each band represents the arbitrary densitometry units of the corresponding band. The blots shown here are representative of three independent experiments with comparable results. **B,** cell death induced by Taxol was significantly reduced in cells over-expressing γ-synuclein. A2780 and A2780/gam cells treated with Taxol for 48hr in the presence or absence of the MEK1/2 inhibitor, U0126. Cell death was determined by trypan blue staining (shown) and WST-1 assays (not shown). The graph represents the average ± S.E. of three independent experiments. (*) Represents significant difference in cell death between A2780 and A2780/gam cells (p < 0.05).

induced cell death can be suppressed by γ-synuclein over-expression (**Fig. 8B**). When ERK activation was inhibited using the MEK1/2 inhibitor U0126, the cell death was reduced by ~25% in A2780 cells but was nearly doubled in A2780/gam cells (**Fig. 8B**). These data suggest that enhanced cell survival in γ-synuclein over-expressing cells is partially mediated by activation of ERK.

To determine whether the protective role of γ-synuclein on cell survival is also mediated through down-regulating JNK associated apoptotic pathway(s), caspase-3 activity was monitored at different time points after Taxol treatment. Consistent with the data in other ovarian and breast cancer cell lines (Lee et al., 1998; Wang et al., 1999), JNK was activated in A2780 cells when treated with 30 µM Taxol. The caspase-3 substrate PARP was found cleaved in cells treated with Taxol (data not shown). In A2780/gam cells, the activity of JNK was significantly inhibited following treatment with Taxol (p < 0.05) (**Fig. 9A**). In the parental A2780 cells, caspase-3 was highly activated following drug treatment. By contrast, the activation of caspase-3 by Taxol treatment was significantly reduced in γ-synuclein over-expressing ovarian cancer cells (p < 0.05) (**Fig. 9B**). These data indicate that Taxol activated JNK mediated caspase apoptotic pathway was significantly attenuated in cells over-expressing γ-synuclein. Taken together, our results indicate that the cell death in ovarian cancer cells induced by Taxol may be protected by γ-synuclein and that this may be mediated by regulating the JNK and ERK signaling pathways.
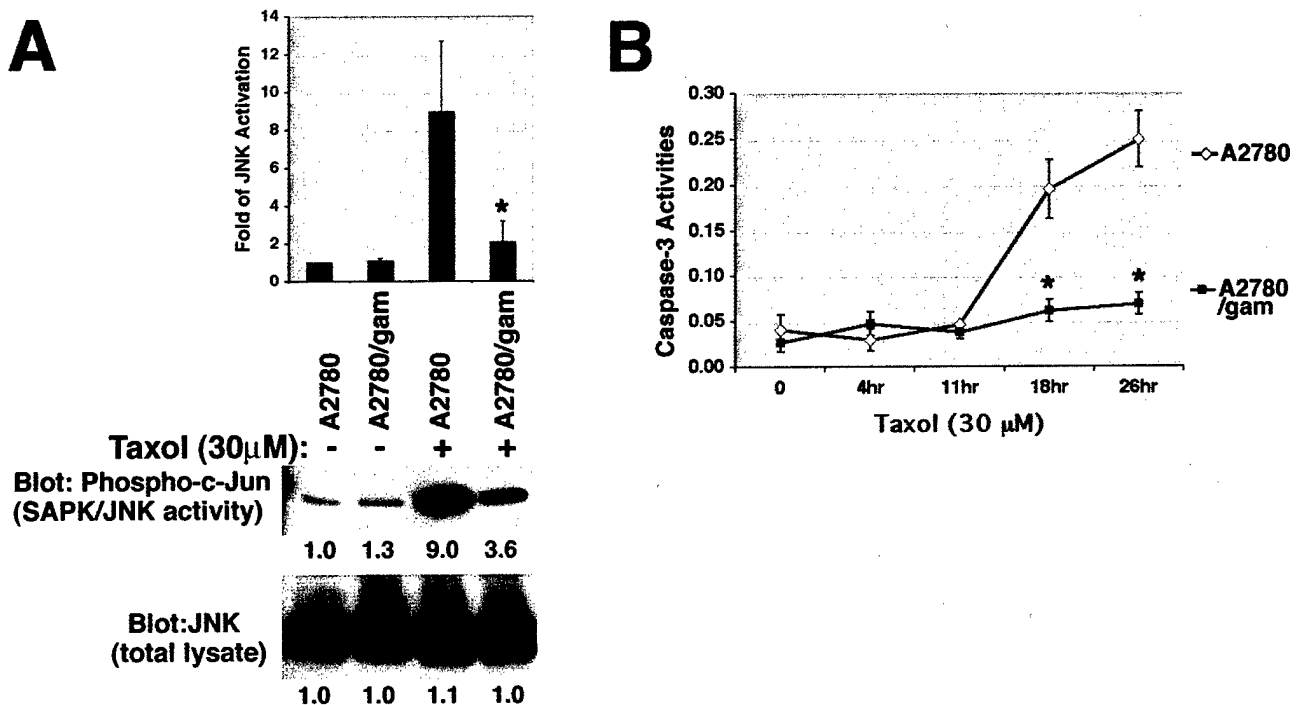
Fig. 9. **Taxol activated JNK and caspase-3 apoptotic pathway is blocked by γ-synuclein. A,** down-regulation of JNK activation by Taxol in cells over-expressing γ-synuclein. Cell lysates from A2780 and A2780/gam cells treated with or without Taxol (30 μM) for 60 min were assayed for JNK activity (see the legend for **Fig. 8** for experimental details). The number beneath each band represents the arbitrary densitometry units of the corresponding band. The blots shown here are representative of three independent experiments with comparable results. JNK activities were measured by levels of phosphorylated c-JUN and normalized to levels of total JNK proteins. The graph above the blots is the average ± S.E. of three independent experiments. **B,** down-regulation of caspase-3 activity activated by Taxol in A2780/gam cells. A2780 and A2780/gam cells treated with Taxol (30 μM) for different time lengths were lysed, and approximately 20 g protein were incubated with the caspase-3 substrate Ac-DEVD-pNA for 4hr at 37ßC. Cleavage of the substrate to release pNA was monitored at 405 nm using a microplate reader. The graph represents the average ± S.E. of three independent experiments. In panels A and B, (*) represents significant difference compared to that in the parental cells ($p < 0.05$).

γ-Synuclein over-expression leads to protection from vinblastine but not etoposide induced cell death.

A

B

Blot: Phospho-c-Jun (SAPK/JNK activity)
1.0 3.4 5.9 3.2 6.9 1.6 2.6 3.6 2.0 3.7

Blot:JNK (total lysate)
1.0 1.0 1.1 1.0 1.1 1.2 0.9 1.1 1.2 1.0

C

Blot: ⓟERK
1.0 2.3 2.4 0 0 2.9 3.1 2.6 0 0

Blot: ERK1/2
1.0 1.1 0.9 1.0 1.1 0.9 0.9 0.9 0.9 1.0

Fig. 10. Vinblastine induced cell death and activation of the MAPK pathways in γ-synuclein over-expressing cells. **A,** the cell death induced by vinblastine was significantly reduced in cells over-expressing γ-synuclein. A2780 and A2780/gam cells treated with vinblastine (0.1, 1, or 10 M) for 30 hr in the presence or absence of the MEK1/2 inhibitor, U0126. Cell death was determined by trypan blue staining. The graph represents the average ± S.E. of three independent experiments. (*) Represents significant difference in cell death between A2780 and A2780/gam cells (p < 0.05). **B,** inhibition of JNK activation by γ-synuclein in response to vinblastine treatment. A2780 and A2780/gam cells were untreated or treated with vinblastine (1μM, and 10μM) for 30 min and 60 min before lysis for JNK activity assay. JNK activities were analyzed as described in the legend for Fig. 5. The number beneath each band represents the arbitrary densitometry units of the corresponding band. The blots shown here are representative of three independent experiments with comparable results. **C,** the effect of vinblastine on ERK phosphorylation. A2780 or A2780/gam cells pre-treated with or without U0126 were treated with vinblastine (1 μM and 10 μM) in the absence or presence of U0126 (10 μM) for 30 min. Whole cell lysates were separated by SDS-PAGE and blotted with phospho-ERK to determine the level of active ERK, or with ERK1/2 antibodies for the level of total ERK1/2. The number beneath each band represents the arbitrary densitometry units of the corresponding band. The blots shown are representative of three independent experiments with comparable results.

To demonstrate whether the effects of γ-synuclein on cell survival were specific to Taxol or were a general mechanism of drug resistance, we evaluated two additional chemotherapeutic agents, i.e., vinblastine and etoposide. Both Taxol and vinblastine are microtubule-interfering agents; Taxol binds to microtubule polymers while vinblastine binds to monomers and dimers. When treated with vinblastine (either 0.1, 1.0, or 10 M for 30 hr), cell death in A2780/gam cells was significantly lower (p < 0.05 for all the three concentrations tested) as compared to the parental cells (**Fig. 10A**). Consistent with other studies using a variety of tumor cell lines (Fan et al., 2000; Osborn and Chambers, 1996; Stone and Chambers, 2000; Wang et al., 1999), vinblastine strongly activate JNK in A2780 cells. However, this activation of JNK by vinblastine was significantly inhibited by γ-synuclein over-expression (**Fig. 10B**). Furthermore, we observe that treatment with vinblastine results in a two-fold increase in phosphorylated ERK1/2 in A2780 cells. This enhancement in activated ERK levels was not observed in the A2780/gam cells (**Fig. 10B**). Unlike our results for Taxol, inhibition of ERK phosphorylation by U0126 did not significantly affect the cell death in the parental cells or A2780/gam cells (**Fig. 10A & C**). These data

indicate that suppression of vinblastine-induced cell death by γ-synuclein may be mediated by inhibition of JNK activation.

We also evaluated etoposide, a DNA damage agent that has also been shown to induce JNK activation in some cell lines (Anderson et al., 1999; Gibson et al., 1999; Jarvis et al., 1999; Osborn and Chambers, 1996). When treated with 1, 10, or 100 M of etoposide for 56 hrs, there was no significant difference in cell survival between A2780 and A2780/gam cells (**Fig. 11A**). As might be predicted, JNK was not activated in response to etoposide treatment (**Fig. 11B**). Furthermore, etoposide treatment did not result in ERK activation in A2780 cells. However, surprisingly the constitutive phosphorylated ERK levels observed in A2780/gam cells were significantly down-regulated within 30 min of treatment with 10 or 100 M of etoposide (**Fig. 11C**). In the presence of the MEK inhibitor U0126, cell death induced by etoposide was reduced in both the parental and γ-synuclein over-expression cells, but statistical analysis indicated these differences were not statistically significant.
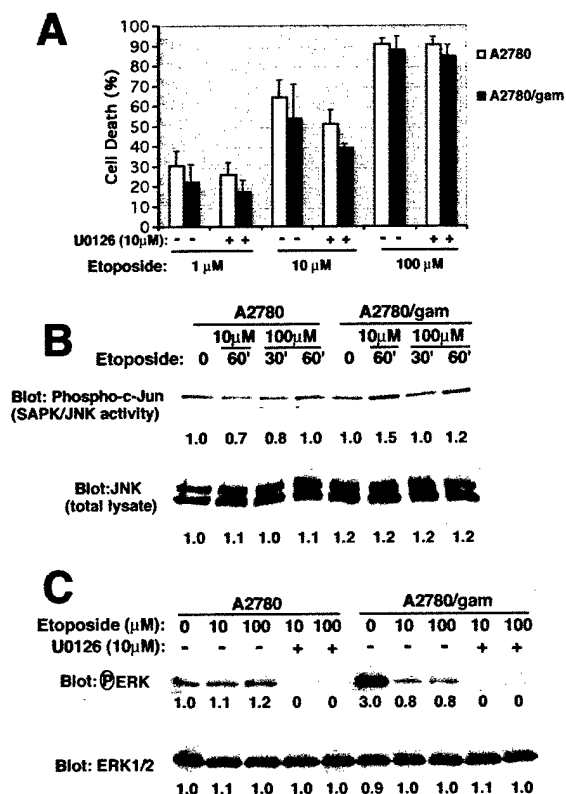
Fig. 11. Effects of γ-synuclein over-expression on etoposide induced cell death and activation of the MAPK pathways. A, cell death induced by etoposide was not significantly altered in cells that over-express γ-synuclein. A2780 and A2780/gam cells treated with etoposide (1 μM, 10 μM, 100 μM) for 56 hr in the presence or absence of the MEK1/2 inhibitor, U0126. Cell death was determined by trypan blue staining. The graph represents the average ± S.E. of three independent experiments. B, vinblastine treatment does not induce JNK activation. A2780 and A2780/gam cells treated with or without etoposide (10 μM, and 100 μM) for 30 min and/or 60 min before lysis for JNK activity assay. JNK activities were analyzed as described in Fig. 5. The number beneath each band represents the arbitrary densitometry units of the corresponding band. The blots shown here are representative of three independent experiments with comparable results. C, effect of etoposide on ERK phosphorylation. A2780 or A2780/gam cells pre-treated with or without U0126 were treated with etoposide (10 μM and 100 μM) in the absence or presence of U0126 (10 μM) for 30 min. Whole cell lysate were separated by SDS-PAGE and blotted with phospho-ERK to determine the level of active ERK, or with ERK1/2 antibodies for the level of total ERK1/2. The number beneath each band represents the arbitrary densitometry units of the corresponding band. The blots shown here are representative of three independent experiments with comparable results.

Summary. Based on the data we presented above, we proposed the following model (**Fig. 12**) that γ-synuclein may enhance cell migration and metastasis, promote cell survival and inhibit apoptosis in tumor progression by modulating Rho and MAPK pathways.
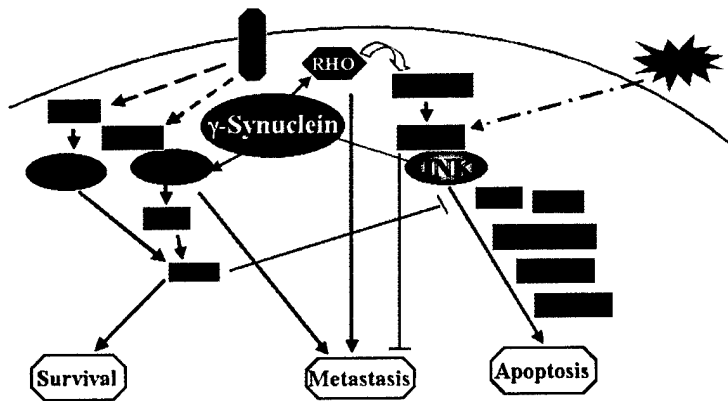
Figure 12. Diagram illustrating the effects of γ-synuclein on relevant signaling transduction pathways and their effects on metastasis, cell survival and apoptosis.

C- KEY RESEARCH ACCOMPLISHMENTS:

C.1. Gamma synuclein promotes a metastatic phenotype in breast and ovarian tumor cells by modulating the Rho signal transduction pathway .

1.a.   Overexpression of γ-synuclein leads to constitutive activation of ERK and Rho/Rac/Cdc42 and down-regulation of JNK activation in response to stress signals or chemotherapy drugs.

1.b.   Overexpression of γ-synuclein induces stress fiber formation and enhances cell migration. Both the basal level and the enhanced cell migration require the activities of both the ERK and Rho/Rac/Cdc42 kinases.

1.c.   Overexpression of γ-synuclein may render cancer cells resistant to Taxol and vinblastine by modulating ERK cell survival pathway and JNK-mitochondria-Caspase 9/3 apoptotic pathway.

1.d.   Published two manuscripts (listed below) and numerous abstracts regarding these studies during the funding period.

1.e.   Received funding from the Army to continue the studies which were supported by this concept mechanism, i.e., Developing inhibitors of ovarian cancer progression by targeted disruption of the γ-synuclein activated migratory and survival signaling pathways , DOD, OC020220.

D-REPORTABLE OUTCOMES (5/2001 to present):

D.1.. Gamma synuclein promotes a metastatic phenotype in breast and ovarian tumor cells by modulating the Rho signal transduction pathway .

*=supported by DAMD17-01-1-0522

1.a. Abstracts

Querec, T.D., Gruver, B.N., Patriotis, P.C., Stoyanova, R.S., Frolov, A.E., Engstrom, P.F., **Godwin, A.K.,** Brown, T.R., Patriotis, C. Differential gene expression patterns associated with the in vitro malignant transformation of human ovarian epithelial cells and chemopreventitive treatment with fenretinide. Proceedings of American Association of Cancer Research, 609:3277, 2001.

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E-CONCLUSIONS:

E.1. **Gamma synuclein promotes a metastatic phenotype in breast and ovarian tumor cells by modulating the Rho signal transduction pathway.**

In these studies, we found that γ-synuclein can interact with two major MAPKs, i.e., ERK and JNK1. Over-expression of γ-synuclein may lead to enhanced activity of ERK and down-regulation of JNK activation in response to stress and chemotherapy drugs. Rho/Rac/Cdc42 pathway is also activated in cells over-expressing γ-synuclein. Activation of both the Rho/Rac/Cdc42 and ERK pathways are required for the enhanced cell migration in γ-synuclein over-expressing cells. Over-expression of γ-synuclein may render cancer cells resistant to Taxol and vinblastine by modulating ERK cell survival pathway and JNK-mitochondria-Caspase 9/3 apoptotic pathway. Taken together, these data indicate that γ-synuclein may promote tumorigenesis by enhancing cell motility through modulating Rho/Rac/Cdc42 and ERK pathways, and promoting cell survival and inhibiting apoptosis through modulating ERK cell survival and JNK-mitochondria-caspase9/3 apoptotic pathways. Since γ-synuclein is aberrantly expressed in the majority of late-stage breast and ovarian cancers but is not expressed in normal breast and ovarian epithelial cells, γ-synuclein may represent a very promising therapy target for these diseases. In this aspect we have obtained an idea award from the Army to pursue identifying small proteins that may block its activity.

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G. LIST OF PERSONNEL PAID FROM GRANT

Godwin, Andrew K.

H. APPENDICES

None